Melatonin, the time keeper: biosynthesis and effects in fish

by

Jack FALCÓN* (1), Laurence BESSEAU (1), Elodie MAGNANOU (1),
Maria-Jesus HERRERO (1), Masanori NAGAI (2) & Gilles BOEUF (1, 3)

ABSTRACT. - Melatonin is the time-keeping molecule of vertebrates. The daily and annual variations of its rhythmic production allow synchronizing metabolism, physiological functions and behaviour to the environment changes. In fish, melatonin is produced by the photoreceptors of the pineal organ and retina as well as by other retinal cell types in the inner nuclear and ganglion cell layers. In most species, the melatonin rhythm displays a high-at-night profile, resulting from the circadian control of the arylalkylamine N-acetyltranferase (AANAT) activity. AANAT is the penultimate enzyme in the melatonin biosynthesis pathway. This review summarizes our current knowledge on the molecular and cellular mechanisms controlling the rhythmic production of melatonin, as well as on the targets and modes of action of the hormone in fish, with special emphasis on neuroendocrine functions.

RÉSUMÉ. - La mélatonine, l'hormone "donneuse de temps" : biosynthèse et rôles chez les poissons.

La mélatonine est la molécule donneuse de temps des vertébrés. Les variations journalières et annuelles de sa production rythmique permettent la synchronisation des métabolismes, fonctions physiologiques et comportements sur les changements de l'environnement. Chez les poissons, elle est produite par les photorécepteurs de l'organe pinéal et de la rétine de même que par des cellules rétiniennes des couches nucléaire interne et ganglionnaire. Chez la plupart des espèces étudiées, les taux de mélatonine sont élevés de nuit et faibles de jour. Ce rythme résulte du contrôle par des horloges circadiennes de l'activité arylalkylamine N-acétyltranférase (AANAT), l'avant-dernière enzyme de la voie de synthèse de la mélatonine. Ici sont résumées les connaissances actuelles sur les mécanismes moléculaires et cellulaires du contrôle rythmique de la production de mélatonine, ainsi que sur les cibles et modes d'action de l'hormone chez les poissons, en particulier sur les fonctions neuroendocrines.

Key words. - Pineal organ - Retina - Pituitary - Melatonin - Arylalkylamine N-acetyltranferase - Biological clocks.

From the molecule to the cell, from the organ to the organisms, and also from organisms to populations, all the activities of living organisms are rhythmic. In a great majority of cases, these rhythms are synchronized to the daily and annual variations of light and temperature. In fish, photoperiod is the main factor that synchronizes daily activities, including locomotor activity, schooling behaviour, sedation, skin pigmentation, food intake, oxygen consumption or thermal preference. Other factors, such as temperature also play a key role by modulating these oscillations. The seasonal changes of the external parameters of the environment also contribute to synchronize periodic functions displaying annual rhythms such as growth and reproduction. In order to face these daily and annual variations fish as other living organisms have developed specific captors as well as internal time keeping biological clocks. The so-called circadian clocks (circa = about; dian = day) free-run under constant conditions and allow anticipating the daily changes of photoperiod and temperature, so that the right event will occur at the right time. Similarly, circannual clocks predict annual changes. It is of upmost importance that birth and growth occur at the most favourable periods of the year, in order to ensure an optimal survival. The functional organization of the circannual system is far less understood than the organization of the circadian system (Bradshaw and Holzapfel, 2007; Paul *et al.*, 2008). The latter includes a core clock mechanisms, input pathways to synchronize it and output pathways that inform the organism.

In fish, the retina and pineal organ are full cellular circadian systems; they integrate the photoperiodic and thermal information, and this allows synchronizing the molecular clock machinery that drives the rhythmic production of messengers. Melatonin is one such a signal now considered as the 'time-keeping' hormone or 'needle' of the biological clocks. Indeed, melatonin production by both organs is rhythmic and reflects the prevailing photoperiod. There are however several important differences between these organs. The first difference is that only pineal melatonin is released into the cerebrospinal fluid (CSF) and blood, thus acting as a hormonal messenger for the rest of the organism.

---

(1) UPMC, UMR 7232 BIOM, GDR 2821, Laboratoire Arago, Avenue Fontaulé, F-66650 Banyuls-sur-Mer, FRANCE.
[besseau@obs-banyuls.fr] [magnanou@obs-banyuls.fr] [maria-jesus.herrero@obs-banyuls.fr] [gilles.boeuf@mnhn.fr]
(2) Yamanashi Institute of Environmental Sciences, Division of Environmental Health Sciences, Department of Physiology, 5597-1 Aza-Kenmarubi, Kamiyoshida, Fujiyoshida, Yamanashi, 403-0005, JAPAN. [mnagai@yies.pref.yamanashi.jp]
(3) Muséum national d'Histoire naturelle, 43 rue Cuvier, 75005 Paris, FRANCE.
* Corresponding author [falcon@obs-banyuls.fr]

Cybium 2011, 35(1): 3-18.

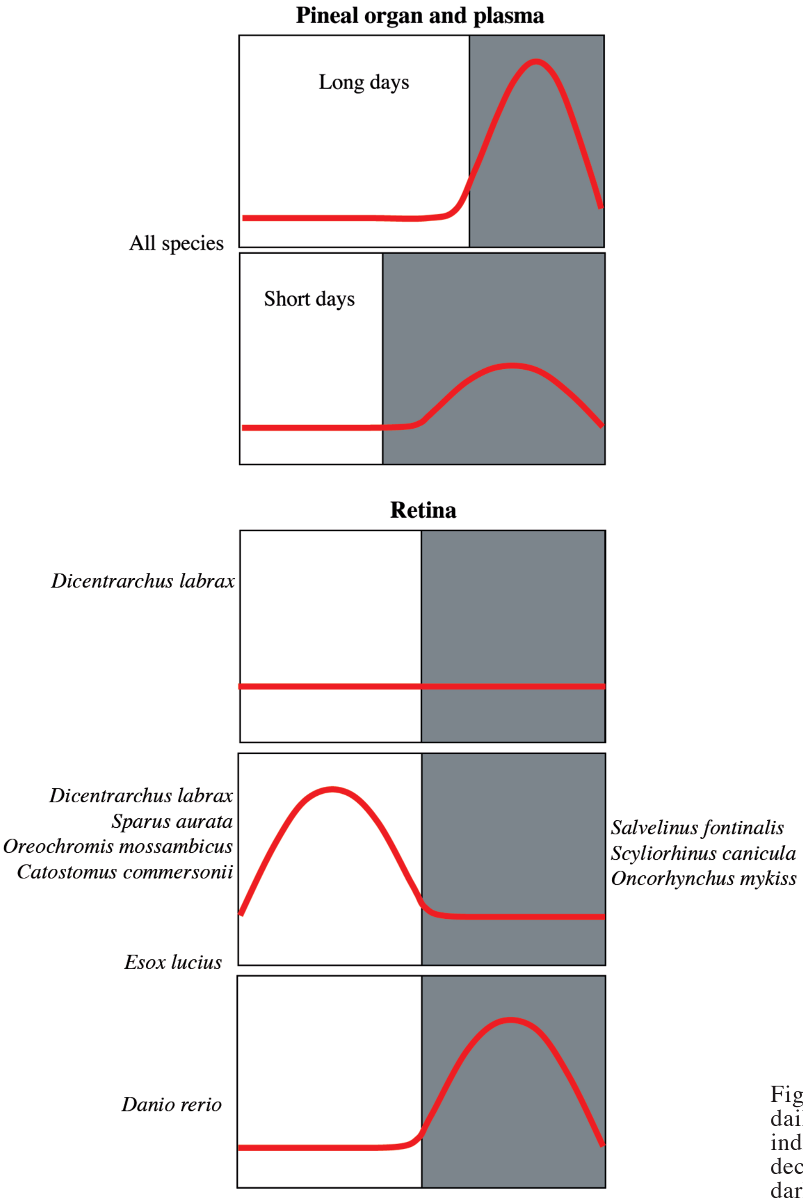

Figure 1. - Melatonin fluctuations. Daily profiles of melatonin content in the pineal organ and plasma under long and short days, and in the retina as a function of species. The position of the names indicates whether the peak is diurnal, nocturnal or intermediate. Grey boxes: darkness.

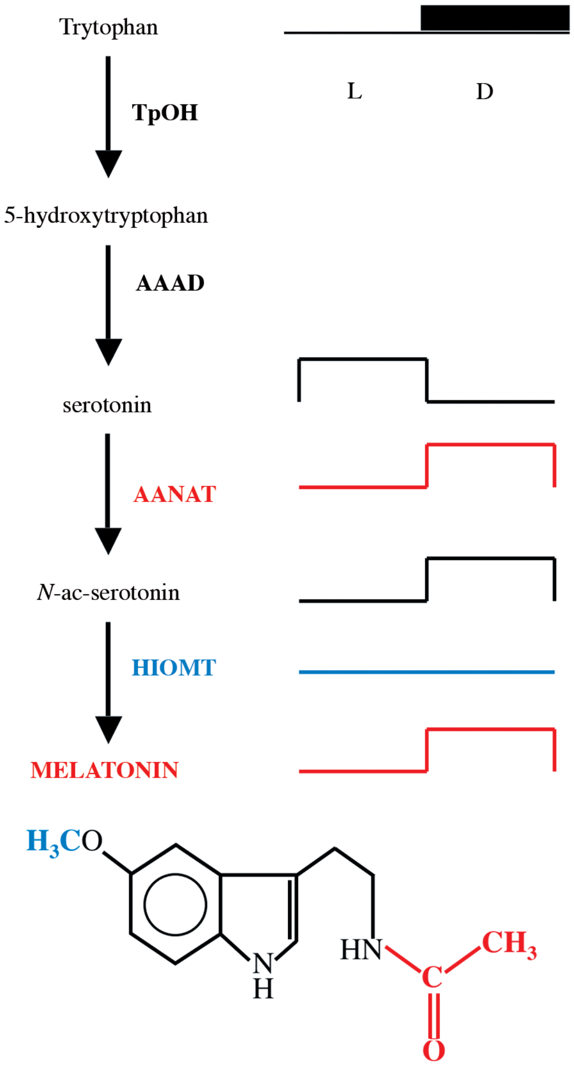

Figure 2. - Melatonin biosynthesis pathway in the pineal. The daily rhythms of the corresponding compounds or enzymes are indicated on the right hand column. AAAD: aromatic aminoacid decarboxylase; AANAT: arylalkylamine N-acetyltransferase; D: dark (black box); HIOMT: hydroxyindole-O-methyltransferase; L: light; TpOH: tryptophane hydroxylase. Modified from Falcón *et al*., 2007a.

Conversely, retinal melatonin is a local (autocrine/paracrine) modulator, which is eventually metabolized locally. Another difference lies in the pattern of melatonin production. The production by the pineal organ is exclusively nocturnal (Fig. 1): its duration reflects the duration of the night and its amplitude depends on the ambient temperature. In contrast, retinal melatonin may be produced at night, or during the day or at the inter-phases, depending on the species or, within the same species, on the season (Fig. 1). Whatever it is, melatonin is a perfect indicator of the time of the day and year. The present review deals with the processes that control the rhythmic production of melatonin by the pineal gland and retina; it provides indication that other central and peripheral tissues might also be producing the hormone in fish. We finally discuss the modalities through which melatonin impacts on fish physiological regulations.

MELATONIN BIOSYNTHESIS PATHWAY

The melatonin biosynthesis pathway starts with the hydroxylation of tryptophan catalyzed by the tryptophan

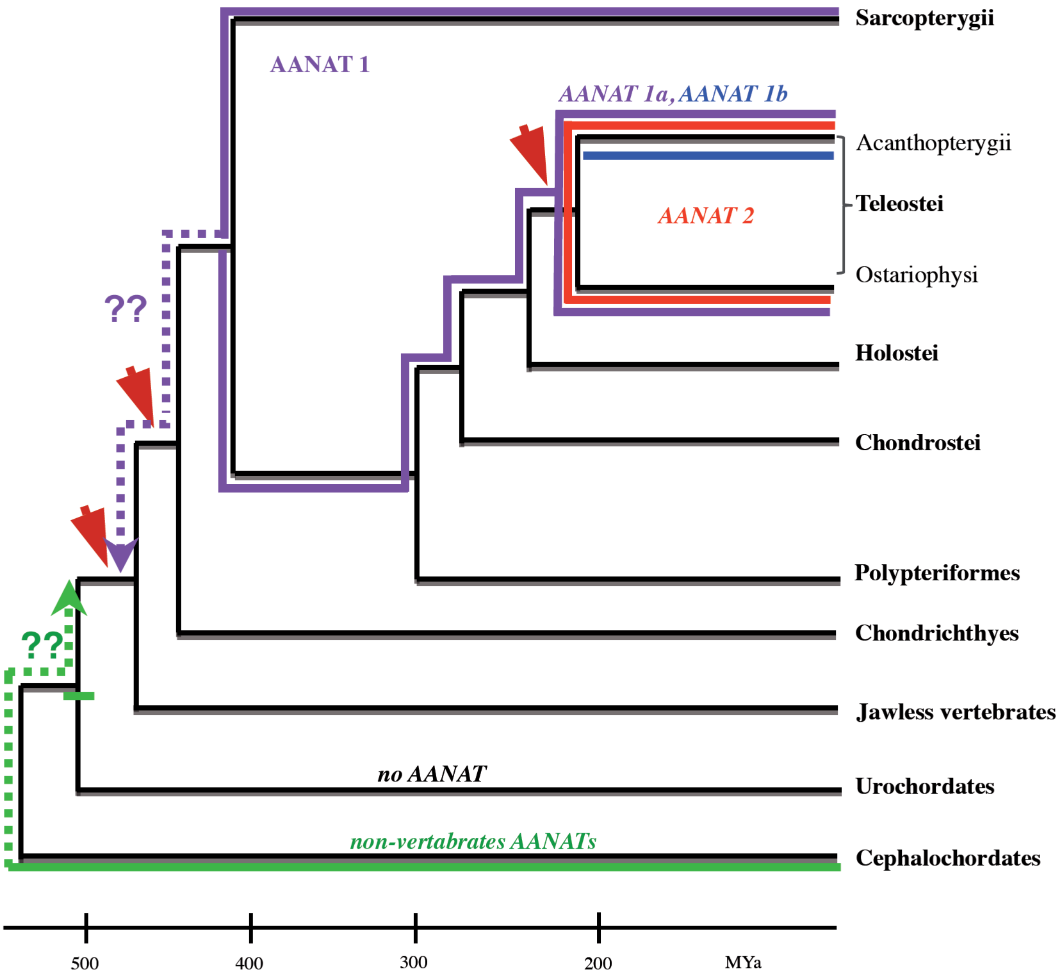

Figure 3. - Distribution of the arylalkylamine N-acetyltransferases in prechordates and vertebrates. Arrows indicate whole gene duplication events. AANAT evolution hypothesis tree was mapped on a phylogenetic tree adapted from Ravi and Venkatesh (2008) and Santini *et al.* (2009).

hydroxylase (TPOH). The 5-hydroxytryptophan is then decarboxylated by the aromatic amino-acid decarboxylase (AAAD) to produce serotonin. The arylalkylamine N-acetyltransferase (AANAT) catalyses the conversion of serotonin to N-acetylserotonin, which is then O-methylated by the action of the hydroxyindole-O-methyltransferase (HIOMT) to produce melatonin (Fig. 2; Klein *et al.*, 1997; Falcón *et al.*, 2007a, 2010). Melatonin biosynthesis is only part of the indole metabolism pathway in the pineal organ and retina. Indeed, the oxidative deamination of serotonin by the monoamine oxidase (MAO) can also lead to the formation of 5-hydroxyindole acetic acid and 5-hydroxytryptophol, which may in turn be methylated by HIOMT. Finally, melatonin can be deacetylated *in situ* to produce 5-methoxytryptamine and 5-methoxytryptophol (Falcón *et al.*, 1985a; Yañez and Meissl, 1995).

Teleost fish are unique because they possess at least two forms of AANAT, unlike all other vertebrates, probably as a result of genome duplication (Jaillon *et al.*, 2004) (Fig. 3). The so-called AANAT1 and AANAT2 display tissue specific distribution: AANAT1 is more specifically expressed in the retina and brain, whereas AANAT2 is more specifically expressed in the pineal organ. Recently, the genome analysis of two species of pufferfish (*Takifugu rubripes* and *Tetraodon nigroviridis*) and of the medaka (*Oryzias latipes*) revealed that three AANAT genes are present: AANAT2 and two AANAT1, termed 1a and 1b (Coon and Klein, 2006). This has implications in terms of photic regulation of melatonin production.

THE SITES OF MELATONIN BIOSYNTHESIS

The pineal organ

The first studies dealing with the teleost pineal organ emphasized its role in melatonin production as well as its photoreceptive characteristics, and it soon appeared it was involved in the control of functions and behaviours displaying daily rhythms (for reviews see: Ekström and Meissl, 1997; Falcón, 1999; Falcón *et al.*, 2007b, 2010). The organ is an evagination of the roof of the diencephalon, which usually resembles a vesicle sometimes large enough to cover the whole cerebral hemispheres and olfactory bulbs (Fig. 4). The lumen of the organ communicates with the 3rd ventricle, although this might not be always the case (Omura and Oguri, 1969). It is surrounded by fenestrated blood vessels that do not enter the epithelium. It lacks a blood-brain barrier, so that the epithelium is exposed to the haemal environment in its basal part, and to the cerebrospinal fluid in its apical part (Falcón, 1979; Omura *et al.*, 1985). The pineal vesicle is located in a pit where the skull is usually thinner and translucent, and the skin that covers it is less pigmented (Fig. 4); all this points to adaptations that favour light entry. This is observed even in fish as big as the Atlantic bluefin tuna *Thunnus thynnus*, which brain is located deep below the skull with a cartilaginous tube driving the pineal vesicle up to the skull.

The pineal epithelium contains photoreceptors cells that resemble the retinal cone photoreceptors, both on a structural and a functional point of view. They possess the machinery for the phototransduction cascade, including the chromophores and different types of opsins, transducin, arrestin, and a cyclic nucleotide-gated (CNG) channel operated by cyclic GMP (cGMP); also, the lipid environment is similar, and different from other neuronal and non-neuronal structures, with a particularly rich polyunsaturated fatty acids (PUFA) environment (Henderson *et al.*, 1994; Ekström and Meissl, 1997; Falcón, 1999; Decressac *et al.*, 2002; Falcón *et al.*, 2007a). Light capture by the photopigment molecules eventually results into cell hyperpolarization. One particularity of the pineal cone is that the kinetics of the response to light are slower compared to the retinal cone (Meissl and Ekström, 1988); and, the membrane potential reflects the ambient level of illumination in the pineal. The photoreceptors establish synaptic contacts with ganglion cells (or second order neurons) that send their axons to the brain, through a pineal nerve tract (Fig. 4). Interstitial cells make the third main cell type of the pineal epithelium, which so resembles a simplified retina. The neurons that leave the pineal organ convey an electrical message at night that results from the release of an excitatory neurotransmitter from the depolarized photoreceptor cells. Light induced hyperpolarization of the photoreceptor inhibits the release of the neurotransmitter and thus the discharges of the pineal neurons. The response is intensity dependent over a wide range (6 to 9 log units; Falcón and Meissl, 1981).

In addition to the excitatory neurotransmitter, the photoreceptors also produce melatonin because the compounds (tryptophan, serotonin and melatonin) and the enzymes mRNA and/or proteins (AANAT, HIOMT, MAO) of the indole metabolism appear located in these cells (Falcón and Collins, 1985; Falcón *et al.*, 1985b, 1994). As mentioned before, the pineal organ photoreceptors express mainly Aanat2; *in vivo* and *in vitro* transient expression analyses indicated that the Aanat2 promoters contain the necessary elements to drive expression in the pineal gland (Appelbaum *et al.*, 2004; Zilberman-Peled *et al.*, 2007).

The retina and the brain

The morphofunctional analogies between the pineal and retinal photoreceptors, have led to believe that retinal melatonin biosynthesis occurred in photoreceptor cells. Indeed, both Aanat and Hiomt are expressed in the outer nuclear layer (ONL; Besseau *et al.*, 2006). However, the same study also revealed co-expression of both enzymes in at least two other cells types, one in the basal inner nuclear layer (INL), the other in the ganglion cell layer (GCL), supporting a previous study showing that a melatonin-like compound was present in both the ONL and the INL. All these cell types express Aanat1 (or Aanat1a and Aanat1b) not Aanat2 (Besseau *et al.*, unpubl. data). These data suggest, although they do not prove definitively, that melatonin biosynthesis is not a specific property of photoreceptor cells. In this regard, it is noteworthy that regulation of melatonin secretion differs substantially in the fish retina compared to the vertebrate pineal gland. Indeed, if a nocturnal pattern of melatonin secretion is seen in some species, in others the peak is diurnal or changes throughout the season (Falcón *et al.*, 2010; Fig. 1). Retinal melatonin is not released but rather is acting in an autocrine/paracrine manner.

Interestingly the Aanat1 family of genes is also expressed in some discrete brain areas, either alone or together with Hiomt. This includes the telencephalon, diencephalon (and particularly the preoptic nuclei [NPO]), optic lobes and cerebellum. The pituitary gland also expresses AANAT (authors unpubl. data).

THE REGULATION OF MELATONIN BIOSYNTHESIS BY THE PINEAL ORGAN

Nocturnal melatonin production

Melatonin is produced in a rhythmic manner by both the pineal organ and retina (Falcón *et al.*, 2007a, 2009). In both cases the synthesis is controlled by the environmental LD cycle. This is achieved through controlling the rhythmic activity of AANAT, as is the case in all vertebrates investigated so far (Klein *et al.*, 1997). However, there are noticeable differences between these organs. Pineal AANAT activity and melatonin content increase at night and decrease during the day, and plasma melatonin levels fluctuate as in the pineal because the hormone is highly lipophylic and crosses easily the cell membrane. These daily rhythms also fluctuate in a seasonal manner with the duration being short under long photoperiod and long under short photoperiod (Fig. 1; Kezuka *et al*., 1988; Masuda *et al*., 2003; Vera *et al*., 2007; Strand *et al*., 2008; Seth and Maitra, 2010); the amplitude also changes on an annual basis but variations depend on the species investigated. Thus, the melatonin signal, which reflects the prevailing photoperiod, provides the animal with accurate information on daily and calendar time.

In vitro, the release of melatonin by fish pineal glands or photoreceptor cells cultured under a LD cycle reflects the pattern observed *in vivo* in the pineal organ and plasma (Bolliet *et al*., 1997; Falcón *et al*., 1992, 2007a) (Fig. 5). And, unexpected light at night decreases pineal melatonin content and release, both *in vivo* and *in vitro*. The amplitude of the response to light is proportional to the intensity of the stimulus (Zachmann *et al*., 1992; Max and Menaker, 1992; Bolliet *et al*., 1995; Bayarri *et al*., 2002). Spectral sensitivity curves indicate melatonin production is highly sensitive to blue and green wavelengths (Max and Menaker, 1992; Bayarri *et al*., 2002), as is the case for the nervous message. Beside this general scheme, studies indicate that in some species the control by light may also rely on light perceived through retina, either partially of even exclusively (Migaud *et al*., 2007; Martinez-Chavez *et al*., 2008, Martinez-Chavez and Migaud, 2009; Nikaido *et al*., 2009; Fig. 6). The modalities of the regulation of the pineal melatonin secretion by light caught by the retina remain to be elucidated. In mammals, the photic information perceived through the eyes is conveyed through a retino-hypothalamic tract (RHT), to the suprachiasmatic nuclei of the hypothalamus (SCN), where the master circadian clocks reside; from there, a multisynaptic pathway (hypothalamic paraventricular nuclei [PVN] preganglionic neurons of the sympathetic nervous system superior cervical ganglion [SCG]) connects the SCN to the melatonin producing units of the pineal gland (Simonneaux and Ribelayga, 2003). In birds, both the direct (pineal) and indirect (retinal) pathways mediate the effects of light on the pineal melatonin secretion. It is possible that the situation observed in some teleost fish species reflects the existence of a convergent evolution with tetrapods. The large anatomical diversity observed in the pineal organ of fish as well as the presence, in some species, of more or less "rudimentary" photoreceptors, pinealopetal innervations or catecholaminergic control of melatonin secretion (see below), might be pieces of this "evolutionary puzzle" (Falcón *et al*., 2010).

Another interesting feature observed in most teleost fish investigated is the presence of a photoreceptor cell circadian clock system (Bayarri *et al.,* 2004a; Bolliet *et al*., 1995, 1996, 1997; Cahill, 1996; Iigo *et al*., 1994; Molina Borja *et al*., 1996; Okimoto and Stetson, 1999; Oliveira *et al.,* 2008, 2009), which allows maintaining a rhythmic melatonin output even under constant darkness (DD), with a period that approximates 24 h (Fig. 5). In culture, the shape of the circadian oscillations depends very much on the cell density in the wells. Too high or too low densities induce rapid dampening, which could result from uncoupling between the cellular circadian units due to lack of cellular communication in the first case, or accumulation of some uncoupling agent in the second case. Because melatonin biosynthesis is inhibited by illumination, the endogenous rhythm is not observed under constant light (LL). Salmonid fish, as well as tilapia (*Oreochromis mossambicus*) and sole (*Solea senegalensis*), are exceptions because melatonin oscillations cannot be detected under DD (Gern and Greenhouse, 1988; Thibault *et al*., 1993; Iigo *et al*., 2007; Nikaido *et al*., 2009; Oliveira *et al*., 2009). The absence of circadian oscillations in these species could results from a dysfunction of the molecular clock, or of the pathway linking the clock to the output studied.

Irrespective of the strategies that regulate melatonin secretion, one common feature is that all regulations involve the AANAT as detailed below. The rhythm in melatonin biosynthesis results from the rhythmic activity of AANAT2. Both, the rhythms of AANAT activity and melatonin production can be superimposed *in vivo* or *in vitro*. In contrast, HIOMT activity remains rather constant throughout the LD cycle (Falcón *et al*., 1987; Morton and Forbes, 1988).

Several arylalkylamine N-acetyltransferases (AANAT) in teleost fish

The cloning of fish AANATs soon indicated that teleost fish are unique among vertebrates because they express two or even three AANAT genes that define two main families (Falcón *et al.,* 2007a, 2009) (Fig. 3). It is believed that a first round of whole genome duplication at the base of the teleost fish lineage is at the origin of the AANAT1 and AANAT2 families of enzymes (see above). The appearance at some point of the teleost fish evolution, of two AANAT1 (AANAT1a and AANAT1b) remains yet unexplained (Coon and Klein, 2006). This situation is unique among vertebrates. Most interestingly, the different AANAT families exhibit tissue specific expression. Thus, AANAT2 is specifically expressed in the pineal organ; it has no equivalent in other vertebrate classes. The AANAT1a and AANAT1b enzymes are expressed in the retina and discrete brain areas; AANAT1a and AANAT1b are more similar to the AANATs found in other vertebrates. Studies in the zebrafish indicated that two regions of the Aanat2 gene allow the pineal specific expression; one is located in the 5'-flanking region, and the other 6 kb downstream the transcribed region (Appelbaum *et al*., 2004). The encoded AANAT1 and AANAT2 proteins have distinct affinities for serotonin and differ in their relative affinities for indole-ethylamines versus phenyl-ethylamines (Falcón *et al*., 1996a; Coon *et al*., 1999; Benyassi *et al*., 2000; Zilberman-Peled *et al*., 2004).

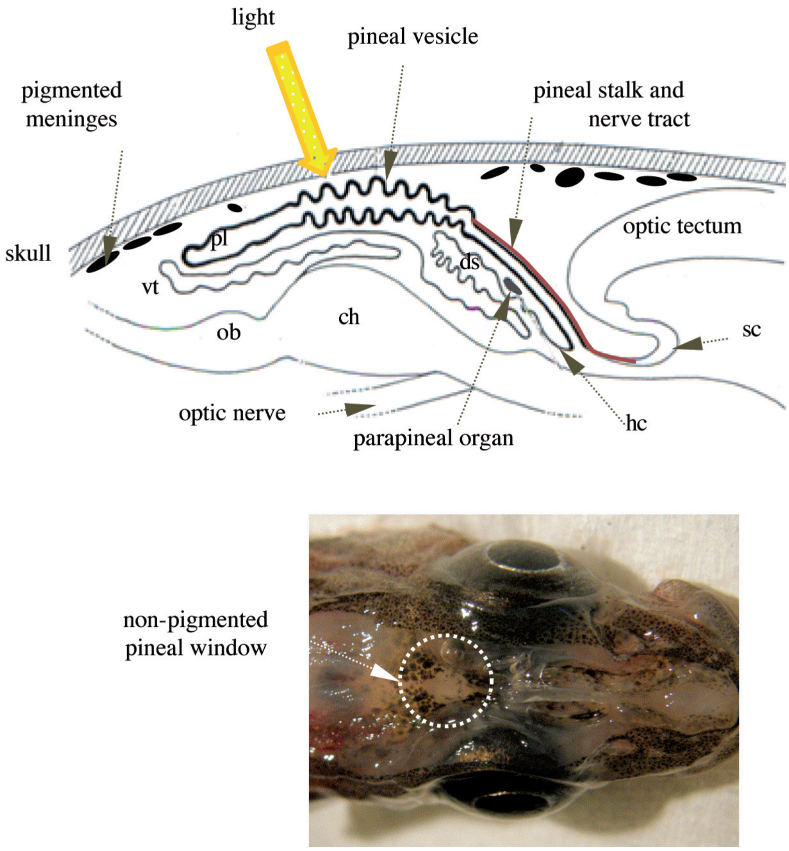

Figure 4. - The fish pineal organ. Above: schematic presentation of a sagittal section through the pike (*Esox lucius*) brain showing the pineal vesicle above the cerebral hemispheres (ch) and olfactive bulbs (ob). The pineal stalk, containing the nervous tract, connects to the brain at the level of the upper diencephalon, between the habenular commissure (hc) and subcommissural organ (sc). ds: dorsal sac; pl: pineal lumen; vt: velum transversum. Adapted from Falcón, 1979. Below: dorsal view of the head of a polar cod (*Boreogadus saida*) showing the depigmented area around the pineal window (in the circle).

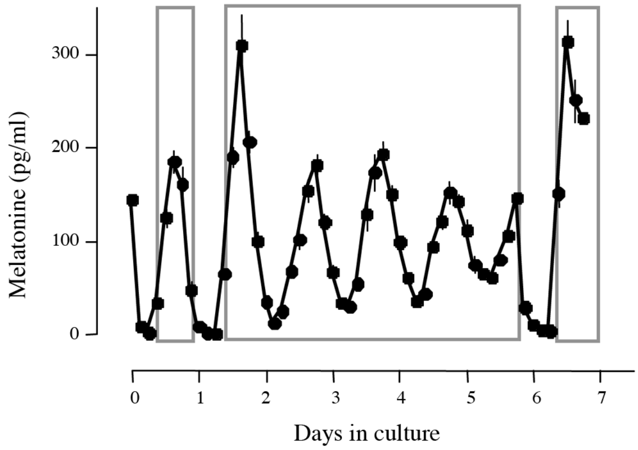

Figure 5. - Circadian production of melatonin by pike (*Esox lucius*) cultured photoreceptor cells *in vitro*. From Bolliet *et al*., 1997, with publisher's permission. Grey boxes: darkness.

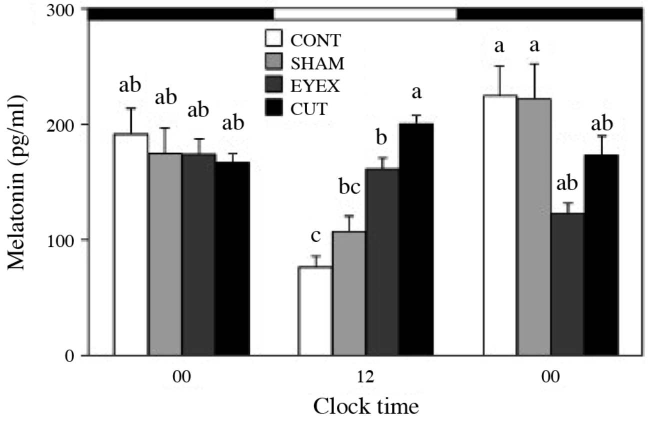

Figure 6. - Light/dark variations of plasma melatonin content in the tilapia (*Oreochromis mossambicus*). The daily rhythm is observed in control (CONT) and sham-operated (SHAM) animals; it is no longer observed in eyectomized (EYEX) or optic trac sectioned (CUT) fish. From Nikaido *et al*., 2009, with publisher's permission.

The arylalkylamine N-acetyltransferase2 (AANAT2), the enzyme of the melatonin rhythm

Regulation of pineal AANAT2 operates at the transcription, traduction and post-traduction levels (Fig. 7). In most of the fish species investigated, AANAT2 transcripts abundance varies, under control by the photoreceptor circadian clock (Bégay *et al*., 1998; Coon *et al*., 1999; Gothilf *et al*., 1999). This rhythm is 3-4 h phase advanced compared to the rhythms in AANAT protein amount and activity. How the circadian time-keeping mechanism operates and is entrained by light is beyond the scope of this review (see Cahill, 2002; Korf *et al*., 2003; Sato *et al*., 2004). We shall just recall that the machinery is based on a molecular feed-

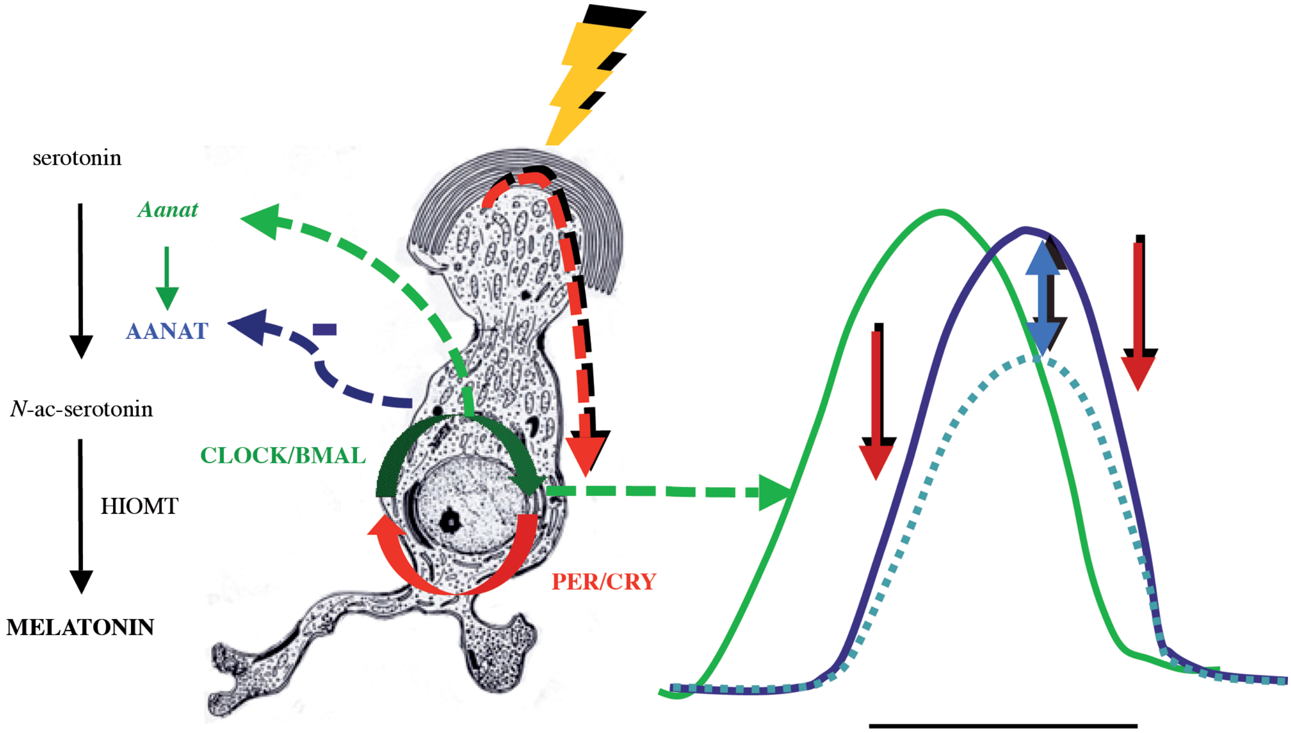

Figure 7. - Schematic presentation of the control of melatonin production in the fish pineal photoreceptor cell. Morning light (yellow arrow) synchronizes the activity of the molecular circadian clock via induction of Per. In turn, the CLOCK/BMAL dimer induces expression of the Aanat2 gene (yellow arrows), which in turn induces production of the AANAT2 protein. Protein accumulates only at night because light induces AANAT2 protein degradation (blue arrows). Temperature modulates the amplitude of the nocturnal surge (double headed arrow). For details see text.

back loop consisting of two heterodimers, PER/CRY acting as repressors, and BMAL/CLOCK acting as activators, and additional interlocking loops. Several copies of each actor exist in fish; thus, in zebrafish four Per, six Cry, three Clock and three Bmal genes have been cloned (Cahill, 2002; Hirayama *et al.*, 2003; Vallone *et al.*, 2004). Light resets the circadian clock, through a morning induction of Per2 in the zebrafish (Ziv *et al.*, 2005), and this light-induced expression determines the phase of the oscillations (Vuilleumier *et al.*, 2006) (Fig. 7). The subsequent later induction of the CLOCK/BMAL heterodimer initiates transcription of the Aanat2, through responsive elements in the gene regulatory sequence (Appelbaum *et al.*, 2004, 2005; Appelbaum and Gothilf, 2006; Zilberman-Peled *et al.*, 2007). The de novo synthesis of AANAT protein is paralleled by an increase in activity and a rise in melatonin production but only late in the evening, when light intensity decreases and at night. A key regulator in this response is intracellular calcium (Ca^{++}), which accumulates in the dark and is depleted upon illumination as a result of photoreceptor depolarization and hyperpolarization, respectively (Falcón *et al.*, 1992, 2001). It is believed that a Ca^{++} induced AANAT2 phosphorylation protects AANAT2 from degradation; this involves both cAMP-dependent and cAMP-independent mechanisms (Falcón, 1999). Upon dephosphorylation AANAT2 is degraded through the proteasome.

Non-photic regulation of arylalkylamine N-acetyltransferase-2 (AANAT2) and melatonin secretion

There is to date no evidence indicating that other factors than photoperiod act on the phase of the rhythms of AANAT2 activity and melatonin secretion. However, external and internal factors may contribute to modulating the amplitude of the nocturnal increase. The main external factor is temperature, which fluctuates on a daily and seasonal basis (Fig. 7). The effects of temperature are species dependent and there is a good correlation between the peak of activity and the fish optimal physiological temperature. Temperature acts directly on the pineal gland through yet unidentified mechanisms. Good candidates could be membrane sensors that control Ca^{++} entry in photoreceptors (Seebacher and Murray, 2007; Myers *et al.*, 2009). Another possible target is the AANAT2 protein itself; indeed, studies on recombinant enzymes have shown distinct responses of the AANAT2 expressed proteins to temperature (Falcón *et al.*, 1996a; Coon *et al.*, 1999; Benyassi *et al*., 2000; Zilberman-Peled *et al*., 2004). In contrast, AANAT1 enzymes respond similarly to temperature with a maximum of activity at 37°C. It is possible that specificities in the one- and three-dimensional structures of the AANAT2 proteins determine the response to temperature by affecting the enzyme kinetics. Whatever it may be, any change in temperature, related to husbandry conditions or global warming, may have dramatic consequences on the time-keeping system of fish.

The role played by internal factors in the control of melatonin production has received little attention. The data available are limited to few fish species, so that no general rule can be extrapolated (review in Falcón *et al*., 2007a). These factors include hormones, neuromodulators and neurotransmitters. The hormones that modulate melatonin secretion are melatonin itself and steroids. Because melatonin receptors have not been identified in the fish pineal the effects are probably the result of an interference with some metabolic pathway. In contrast, receptors to 17-ß-estradiol and glucocorticoids are expressed in the trout pineal gland, which mediate inhibition of AANAT2 (cortisol) and melatonin production (cortisol and 17-ß-estradiol) at night (Bégay *et al*., 1993, 1994; Benyassi *et al*., 2001). A few studies have shown the involvement of endogenous neuromodulators (adenosine, GABA) and neurotransmitters (catecholamines) in the control of melatonin secretion (Ekström *et al*., 1987; Falcón *et al*., 1988, 1991, 1992; Meissl *et al*., 1993, 1994). The existence of a catecholaminergic control in the pineal gland of some fish but not in others indicates there is diversity in the strategies developed by teleost to control melatonin secretion. Whether this is related to the observation that some fish need the eyes to regulate melatonin secretion, while others do not, remains a matter of speculation (Falcón *et al*., 2010).

ROLE OF THE PINEAL ORGAN AND MELATONIN

Melatonin, a time-keeping molecule in fish

Investigations on the role of the pineal organ and melatonin in fish started four decades ago using classical physiological approaches. From these studies, it became obvious that the pineal organ and its hormone contribute to the regulation of behaviour, metabolism and physiology, many of them displaying daily and/or seasonal rhythmic patterns. These include shoaling, locomotor activity, vertical migration, food intake, sedation (sleep), thermal preference, skin pigmentation, modulation of hypothalamic monoamines, plasma and hepatic lipid, glucose and steroids content, $Ca2^+$ metabolism and bone structure, growth, reproduction and perhaps horizontal migration and osmoregulation (Falcón *et al*., 2007b, 2010; Sköld *et al*., 2008) (Fig. 8).

Whatever the topic investigated, a great amount of confusion has always surrounded the studies dealing with the effects of melatonin. Many reasons account for this. One reason is that fish represent a huge amount of species living from temperate to extreme environments, and that a great interspecies variability exists. For example, the role of the pineal in the control of locomotor activity seems to strongly depend on the position of the pineal gland in the hierarchy of organs expressing circadian clock activities because pinealectomy can induce either a total loss of circadian locomotor rhythm, or a change of the period of this rhythm, or fragmentation of the rhythm into several components, depending on the species (Underwood, 1989). Another reason is that melatonin is an output of the circadian clocks, which production reflects the integration of predictable (cyclic), and unpredictable, environmental changes (light and photoperiod, temperature, salinity). As such, melatonin impacts on many functions, including most probably the biological clocks, as is the case in mammals (Falcon *et al*., 2007b). The result is that the effects may vary depending on the time of day or year even within a same species. For example, the rhythm of food intake in the Mediterranean sea bass (*Dicentrarchus labrax*), which is usually diurnal, can switch to nocturnal for unknown reasons (Sanchez-Vazquez *et al*., 2000).

A breakthrough in the understanding of the role melatonin plays in fish was achieved after it became possible to localize the sites of melatonin binding as well as the sites of expression of the genes encoding the melatonin receptors after these were cloned. This is illustrated in the following chapters, which are far from giving an exhaustive view on our current knowledge on the effects of melatonin in fish. For more details, the reader is directed to read the reviews of these latest years (Boeuf and Falcón, 2002; Soengas and Aldegunde, 2002; Falcón *et al*., 2007b, 2010; López-Olmeda and Sánchez-Vázquez, 2010; Migaud *et al*., 2010; Oliveira and Sánchez-Vázquez, 2010; Reiter *et al*., 2010).

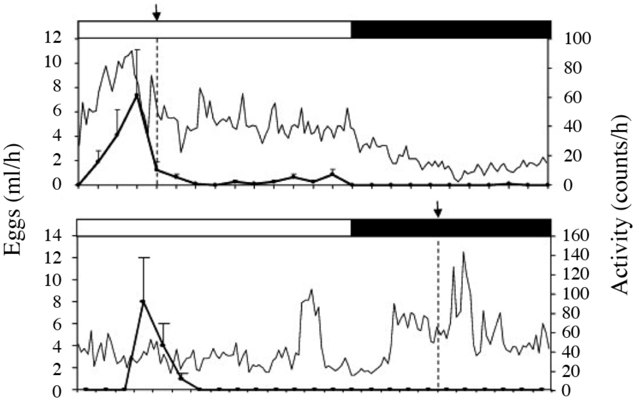

Figure 8. - Daily locomotor activity and spawning rhythms in zebrafish. Under a 14L:10D (black bar) photoperiod, fish display a daily rhythm in locomotor activity and spawning. Feeding time (arrow) affects the former not the latter. From Blanco-Vives and Sánchez-Vázquez (2009) with publisher’s permission.

Melatonin receptors

Studies reporting the cloning, expression and regulation of melatonin receptors remain anecdotic when compared to those dealing with 125I-[2-iodo]-melatonin (125IMel) binding (Boeuf and Falcón, 2002). From the cloning experiments, at least three high affinity receptor subtypes have been identified, the MT1, MT2 and Mel1c receptors, which belong to the seven transmembrane-domain-G-protein-coupled family of receptors (Gaildrat *et al*., 2002; Park *et al*., 2007a, 2007b; Sauzet *et al*., 2008; Confente *et al*., 2010; Herrera-Pérez *et al*., 2010).

The high affinity receptors display a widespread distribution in the brain (including central nervous system and pituitary gland), retina and peripheral tissues (including gills, kidney, liver, intestine, skin and gonads) (Falcón, 1999; Falcón *et al*., 2007b, 2010; Kulczykowska *et al*., 2006; Confente *et al*., 2010; Herrera-Pérez *et al*., 2010). In the brain, gene expression or 125IMel binding is associated with areas that receive or integrate information from sensory organs. These include the olfactive bulbs, pretectal area, glomerular complex, optic tectum, torus longitudinalis, and thalamus. A conspicuous expression is also detected in neuroendocrine regions including the ventral telencephalon, preoptic area (NPO), and hypothalamus. And, melatonin receptors are also evident in the ganglionic cell layer of the cerebellum as well as in the hindbrain, in particular in the oculomotor and trigeminal nuclei and the reticular formation. This suggests an important role of melatonin receptors in the mediation of melatonin actions in visual/light integration, mechanoreception, somatosensation, eye-body motor coordination, and integrative and neuroendocrine functions. It is remarkable that the receptors are often associated with areas, which receive innervations from the retina or pineal organ or both as is the case for the NPO (Ekström and Meissl, 1997). This indicates that the NPO, which is involved in the control of pituitary function and thus of important neuroendocrine regulations, integrates light information from various sources. And, it could itself be a photo-detector because it expresses molecules of the visual cascade (opsins, transducin, arrestin; refs in Falcón *et al*., 2007b). It is interesting that there is no melatonin binding sites in the optic tectum, diencephalon and cerebellum of *Coryphaenoides armatus*, a deep-sea teleost fish species that receives no information from solar light (Smith *et al*., 1996). Overall, the number of binding sites in these fish is lower than in surface-dwelling fish, and is restricted to areas associated with chemo- and mechanoperception.

It seems that the impact of melatonin depends not only on its daily and seasonal release, but also on the amount of receptors available at a given time of day or year. This may vary from a species to another and, within a same species, on the tissue investigated or age. Indeed, significant light/dark (LD) variations of 125IMel binding characteristics (Bmax and/or KD) have been reported to occur in whole brain preparations or specific brain areas [e.g., sea bream (*Sparus aurata*), pike (*Esox lucius*), catfish (*Silurus asotus*) and sole (*Solea senegalensis*)] (Falcón *et al*., 1996b; Iigo *et al*., 1997; Gaildrat *et al*., 1998; Oliveira *et al*., 2008); Bmax was observed either during day or at night. No or non-significant variations have been observed in masu salmon (*Onconrhynchus masu*; Amano *et al*., 2006) and sea bass brains (*Dicentrarchus labrax*; Bayarri *et al*., 2004b). Most interestingly, the variations in 125IMel binding observed under LD where maintained under constant LL or DD in pike (Gaildrat *et al*., 1998). Variations in melatonin receptors expression have also been reported in brain areas, with maximum levels of mRNA occurring at night or night-to-day transition in the rabbitfish (*Siganus punctatus*; Park *et al*., 2007a, 2007b), goldfish (*Carassius auratus*; Ikegami *et al*., 2009a), grass puffer (*Takifugu niphobles*; Ikegami *et al*., 2009b), and sole (*Solea senegalensis*; Confente *et al*., 2010). In the diencephalon and optic tectum of the sole and grass puffer, abundance of mRNA corresponding to the melatonin receptors varied on a daily and annual basis. However, the shape of the oscillations varied from one brain area to another. The study in the sole further demonstrated annual variations in MT1, MT2 and Mel1c mRNA abundance as well as amplitude of the daily rhythms (Confente *et al*., 2010) supporting binding data on the sea bass showing seasonal and circannual variations in 125IMel binding in some brain areas (Bayarri *et al*., 2010). This indicates that complex daily and seasonal mechanisms control the expression of melatonin receptors in the fish brain, which may involve circadian and circannual clocks. The localization of these clocks remains to be elucidated; the fact that 125IMel binding remained rhythmic in the brain of pike maintained under constant light indicates pineal melatonin is not necessarily involved in this circadian control. In addition to light and circadian clocks, temperature and salinity are likely to impact melatonin binding (López Patiño *et al*., 2008; Lopez-Olmeda *et al*., 2009; Falcón and Boeuf, unpubl. data). In addition to the brain, other tissues such as the retina and pituitary also displayed daily and annual variations (review in Confente *et al*., 2010).

Melatonin and the brain pituitary axis

The fish NPO occupies a pivotal position in the network of photo-neuroendocrine regulations (Falcón *et al*., 2007b). On the one hand, it integrates photoperiodic information from the retina and pineal organ through a nervous pathway, and from the pineal organ through a hormonal (melatonin) pathway (Fig. 9): (i) the nervous input from the pineal organ provides information on rapids changes in environmental light from above (due to upwards and downwards movements, shadows or changes in water turbidity); (ii) the nervous input from the retina informs on light and shapes perceived from the horizontal plane; and, (iii) melatonin pro-

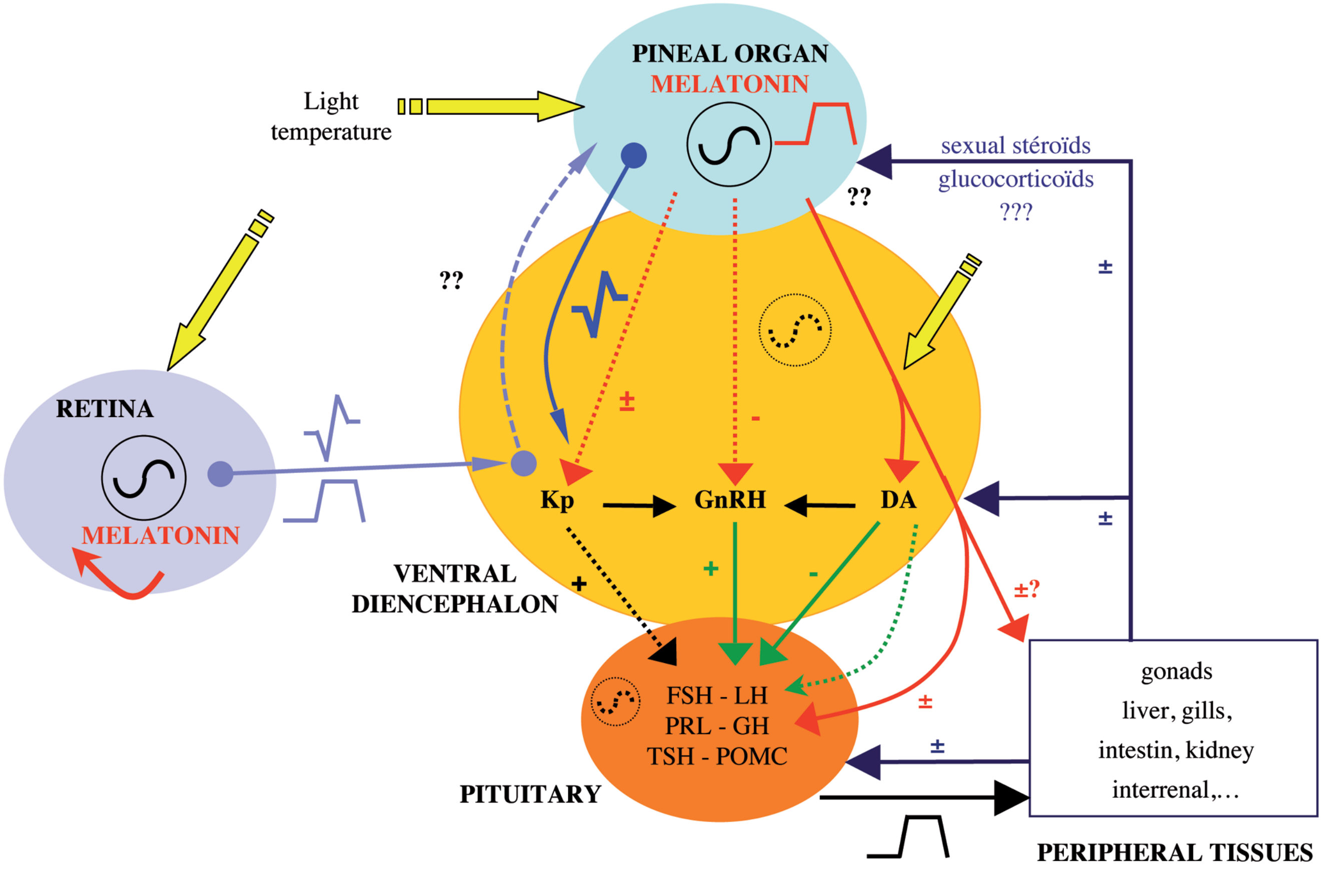

Figure 9. - Photoperiodic and melatonin control of the neuroendocrine axis. The basal diencephalon receives innervations from the retina and pineal organ (blue arrows) two structures containing circadian oscillators synchronized by photoperiod (yellow arrows). Direct light sensitivity and circadian activity might also be present in the brain. In some species a nervous link connects the retina to the pineal gland (interrupted blue arrow; e.g., tilapia).Melatonin from the retina acts locally. Melatonin from the pineal gland is released in the CSF and plasma. In the basal diencephalon it modulates dopamine (DA) release, which contributes to mediate its effects on gonadotropins release by the pituitary gland. Possible effects of melatonin on GnRH and kisspeptin (kp) cells are not excluded. Melatonin also targets the pituitary gland and peripheral tissues. In the pituitary, it modulates GH and PRL release. Possible effects on other pituitary hormones are not excluded.

vides temporal information (day-length and seasonal). On the other hand, neurons from the NPO convey monoaminergic (dopamine, serotonin) and/or peptidergic information to the pituitary gland. The latter corresponds to peptides (isotocine, arginine-vasotocine) that reach the neurohypophysis and releasing factors that control the activity of the pituitary cells, including the pituitary adenylate cyclase peptide (PACAP), and growth hormone (GH) releasing hormone (RH), gonadotropines (Gn)-RH, and corticotrophin-RH. These observations suggest that several of the reported effects of melatonin are mediated through an interaction with the hypothalamic control of pituitary function.

Reproduction

There is, to date, indirect evidence supporting that melatonin might modulate reproduction by targeting the NPO, as suspected for long. However, the mechanisms involved remain unclear. Indeed, melatonin administration induces pro- or anti-gonadal effects in fish or may even have no effect at all. This depends on the species studied, sex, season, dose or way of administration (Zachmann *et al*., 1992; Mayer *et al*., 1997; Falcón *et al*., 2007b, 2010). What appears clear is that melatonin impacts on FSH and LH pituitary production as shown in the Pacific salmon (*Oncorhynchus masou*), where melatonin administration reduced pituitary GnRH and luteinizing hormone (LH) content but stimulated pituitary follicle stimulating hormone (FSH) content (Amano *et al*., 2004). More direct evidence was provided by studies in the Atlantic croaker (*Micropogonias undulatus*), in which intraventricular administration of melatonin in the POA decreased the LH release by the pituitary gland in animals with fully developed gonads (Kahn and Thomas, 1996). Finally, recent studies in goldfish, carp and eel have revealed that melatonin might act on gonadotropins release by modulating the activity of the dopamine system DA (Popek *et al*., 2005, 2006, 2010; Sebert *et al*., 2008; Dufour *et al*., 2010) (Fig. 9).

Growth

It has been known for long that photoperiod controls fish growth, which relies on the secretion of growth hormone (GH) by the somatotrophs of the pituitary gland. This control is a process that involves both stimulatory (e.g., dopamine, thyrotropin releasing hormone [TRH], GH-releasing factor) and inhibitory (e.g., norepinephrine, serotonin, somatostatin [SRIF], GH) agents (see Boeuf and Falcón, 2002 for details). Melatonin may affect GH secretion through controlling upstream regulatory factors, including dopamine (as is the case for reproduction) and other factors as serotonin, in agreement with the observation that melatonin administration modulates dopamine and serotonin hypothalamic content. Melatonin may also target the pituitary gland itself (Falcón *et al*., 2007b, 2010). Melatonin receptors are present in the fish pituitary, and previous conflicting data might result from species-dependent variations or, most likely, from daily and/or seasonal variations in expression of these receptors (Confente *et al*., 2010); this likely renders their detection difficult at some times of the day or year, and easier at others. Indeed, melatonin was shown to modulate *in vitro* cyclic AMP (cAMP) content and GH release at some times of the year but not at others in cultured trout pituitary glands (Falcón *et al*., 2003). The effects of melatonin are complex because daytime (i.e., low) plasma melatonin concentrations induced inhibition, whereas night time (i.e., high) melatonin concentrations induced stimulation, of GH release. In the same species short- or long-term melatonin treatments inhibited similarly pituitary PRL release *in vitro* in a dose-dependent manner. These results are consistent with the observations that plasma GH and PRL levels vary on a daily and seasonal manner in fish (Falcón *et al*., 2003; Fig. 9).

Melatonin and the retina

Binding sites for melatonin have been identified in the fish retina, which were shown to display daily variations in some species, not in others (Bayarri *et al*., 2004b; Amano *et al*., 2006; López Patiño *et al*., 2008; Oliveira *et al*., 2008). Melatonin receptors cloning provided a more accurate view of the receptor subtypes expressed, which also varies from a species to another (Park *et al*., 2007a, 2007b; Sauzet *et al*., 2008; Confente *et al*., 2010). Real time quantitative PCR provided evidence that expression varied on a daily basis in goldfish, rabbitfish, goldfish and sole, excluding Mel1c in the latter (Park *et al*., 2007a, 2007b; Ikegami *et al*., 2009b; Confente *et al*., 2010). The daily variations were under circadian control in the rabbit fish (Park *et al*., 2007a). Usually higher mRNA abundance is seen at night or end of the night. In addition annual variations have also been evidenced. In the sole, the LD variations in MT1 and MT2 receptors expression were observed only in spring, not at the other seasons of the year; and, the overall amount of mRNA abundance also varied differently depending on season and subtype investigated (Confente *et al*., 2010). This would indicate complex actions of melatonin in the retina, which depends on the melatonin secretion profile as well as on the type of receptors expressed at a given time of the day and year. It may also depend on the cell types that express the receptors. Indeed, in situ hybridization studies indicated that the MT1 and MT2 receptors mRNA of the sea bass retina are seen in the ONL, basal INL and GCL (Sauzet *et al*., 2008). Actually, these areas correspond to those where expression of the melatonin biosynthesis enzymes, AANAT and HIOMT, are also seen suggesting melatonin is an autocrine and/or paracrine modulator in the retina (Besseau *et al*., 2006). Receptors of the MT1 and MT2 subtypes are also expressed in the retinal pigment epithelial cells (RPE). The localization in the RPE and different layers of the neural retina support the idea that melatonin is a modulator of visual function as discussed extensively elsewhere (Sauzet *et al*., 2008). In the RPE melatonin might control chemotactic cellular movements, pigment migration and phagocytosis of photoreceptor outer segment membranes. In the neural retina, melatonin controls photoreceptor disk shedding and movements, circadian clock, sensitivity to light, modulation of neurotransmitter release, and more (for details see Ribelayga *et al*., 2004; Ping *et al*., 2008; Sauzet *et al*., 2008).

CONCLUSIONS AND PERSPECTIVES

Melatonin is the key time-keeping hormone in fish where it has multiple effects, including the synchronization of metabolism, physiology and behaviour to the daily and annual variations of the environment. It is produced by the retina and the pineal organ, which share many common properties. In teleost, each organ has, however, its own set of biosynthesis genes, as is the case for the AANAT genes, a unique situation among vertebrates. And, only pineal melatonin is released into the blood stream and CSF. Whereas the mechanisms of melatonin biosynthesis by the pineal organ are quite well understood, the situation is more complex in the retina, where melatonin seems to exert autocrine/paracrine effects related to visual function, and in a species-dependent manner. Also intriguing is the observation that the melatonin enzymes are expressed in different brain areas raising the question of their role. In the pineal gland, the strategies developed by the different fish species all result in a nocturnal surge in melatonin secretion. The photoreceptor cells involved in this production may or may not express a functional circadian clock, and may or may not receive input from the retina. They must be viewed as multi-effectors cells, which activity is modulated not only by photoperiod, but also by temperature, hormones and neurotransmitters. The elucidation of the effects of temperature is a challenge of the forthcoming years, which appears of crucial importance in the context of the global warming. Also a better understanding of how melatonin acts on the fish brain and peripheral organs requires extended, all year long, morphofunctional studies, coupling the identification of the cellular melatonin targets with *in vivo* and *in vitro* pharmacological, physiological and behavioural studies. In this regard, the "omics" approaches should be of great help. These approaches should help elucidating the effects and modes of action of melatonin on reproduction, growth or feeding and may be also unravel other possible targets, including stress and immunity.

REFERENCES

AMANO M., IIGO M., IKUTA K., KITAMURA S., OKUZAWA K., YAMADA H. & YAMAMORI K., 2004. - Disturbance of plasma melatonin profile by high dose melatonin administration inhibits testicular maturation of precocious male masu salmon. *Zool. Sci.*, 21:79-85

AMANO M., IIGO M., KITAMURA S., AMIYA N. & YAMAMORI K., 2006. - Changes in melatonin binding sites under artificial light-dark, constant light and constant dark conditions in the masu salmon brain. *Comp. Biochem. Physiol. A-Mol. Integr. Physiol.*, 144(4): 509-513.

APPELBAUM L. & GOTHILF Y., 2006. - Mechanism of pineal-specific gene expression: the role of E-box and photoreceptor conserved elements. *Mol. Cell. Endocrinol.*, 27(252): 27-33.

APPELBAUM L., TOYAMA R., DAWID I.B., KLEIN D.C., BALER R. & GOTHILF Y., 2004. - Zebrafish serotonin-N-acetyltransferase-2 gene regulation: pineal-restrictive downstream module contains a functional E-box and three photoreceptor conserved elements. *Mol. Endocrinol.*, 18: 1210-1221.

APPELBAUM L., ANZULOVICH A., BALER R. & GOTHILF Y., 2005. - Homeobox-clock protein interaction in zebrafish. A shared mechanism for pineal-specific and circadian gene expression. *J. Biol. Chem.*, 280(12): 11544-11551.

BAYARRI M.J., MADRID J.A. & SANCHEZ-VAZQUEZ F.J., 2002. - Influence of light intensity, spectrum and orientation on sea bass plasma and ocular melatonin. *J. Pineal Res.*, 32: 34-40.

BAYARRI M.J., GARCÍA-ALLEGUE R., LÓPEZ-OLMEDA J.F., MADRID J.A. & SÁNCHEZ-VÁZQUEZ F.J., 2004a. - Circadian melatonin release *in vitro* by European sea bass pineal. *Fish Physiol. Biochem.*, 30: 87-89.

BAYARRI M.J., IIGO M., MUNOZ-CUETO J.A., ISORNA E., DELGADO M.J., MADRID J.A., SANCHEZ-VAZQUEZ F.J. & ALONSO-GOMEZ A.L., 2004b. - Binding characteristics and daily rhythms of melatonin receptors are distinct in the retina and the brain areas of the European sea bass retina (*Dicentrarchus labrax*). *Brain Res.*, 1029(2): 241-250.

BAYARRI M.J., FALCÓN J., ZANUY S. & CARRILLO M., 2010. - Continuous light and melatonin: daily and seasonal variations of brain binding sites and plasma concentration during the first reproductive cycle of sea bass. *Gen. Comp. Endocrinol.*, 169(1): 58-64.

BÉGAY V., VALOTAIRE Y., RAVAULT J.P., COLLIN J.P. & FALCÓN J., 1993. - Photoreceptor cells of the pineal body in culture: effect of 17 beta-estradiol on the production of melatonin. *J. Soc. Biol.*, 187: 77-86.

BÉGAY V., VALOTAIRE Y., RAVAULT J.P., COLLIN J.P. & FALCÓN J., 1994. - Detection of estrogen receptor mRNA in trout pineal and retina: estradiol-17 beta modulates melatonin production by cultured pineal photoreceptor cells. *Gen. Comp. Endocrinol.*, 93: 61-69.

BÉGAY V., FALCÓN J., CAHILL G.M., KLEIN D.C. & COON S.L., 1998 - Transcripts encoding two melatonin synthesis enzymes in the teleost pineal organ: circadian regulation in pike and zebrafish, but not in trout. *Endocrinology*, 139: 905-912.

BENYASSI A., SCHWARTZ C., COON S.L., KLEIN D.C. & FALCÓN J., 2000. - Melatonin synthesis: arylalkylamine N-acetyltransferases in trout retina and pineal organ are different. *Neuroreport*, 11: 255-258.

BENYASSI A., SCHWARTZ C., DUCOURET B. & FALCÓN J., 2001. - Glucocorticoid receptors and serotonin N-acetyltransferase activity in the fish pineal organ. *Neuroreport*, 12: 889-892.

BESSEAU L., BENYASSI A., MØLLER M., COON S.L., WELLER J.L., BOEUF G., KLEIN D.C. & FALCÓN J., 2006. - Melatonin pathway: breaking the "high-at-night" rule in trout retina. *Exp. Eye Res.*, 82: 620-627.

BLANCO-VIVES B. & SÁNCHEZ-VÁZQUEZ F.J., 2009. - Synchronisation to light and feeding time of circadian rhythms of spawning and locomotor activity in zebrafish. *Physiol. Behav.*, 98(3): 268-275.

BOEUF G. & FALCÓN J., 2002. - Photoperiod and growth in fish. *Vie Milieu*, 51: 237-246.

BOLLIET V., FALCÓN J. & ALI M.A., 1995. - Regulation of melatonin secretion by light in the isolated pineal organ of the white sucker (*Catostomus commersoni*). *J. Neuroendocrinol.*, 7: 535-542.

BOLLIET V., ALI M.A., LAPOINTE F.J. & FALCÓN J., 1996. - Rhythmic melatonin secretion in different teleost species: an *in vitro* study. *J. Comp. Physiol. B*, 165(8): 677-683.

BOLLIET V., BÉGAY V., TARAGNAT C., RAVAULT J.P., COLLIN J.P. & FALCÓN J., 1997. - Photoreceptor cells of the pike pineal organ as cellular circadian oscillators. *Eur. J. Neurosci.*, 9: 643-653.

BRADSHAW W. & HOLZAPFEL C., 2007. - Evolution. Tantalizing timeless. *Science,* 316(5833): 1851-1852.

CAHILL G.M., 1996. - Circadian regulation of melatonin production in cultured Zebrafish pineal and retina. *Brain Res.*, 708: 177-181.

CAHILL G.M., 2002. - Clock mechanisms in zebrafish. *Cell Tissue Res.*, 309: 27-34.

CONFENTE F., RENDÓN M.C., BESSEAU L., FALCÓN J. & MUÑOZ-CUETO J.A., 2010. - Melatonin receptors in a pleuronectiform species, *Solea senegalensis*: cloning, tissue expression, day-night and seasonal variations. *Gen. Comp. Endocrinol.*, 167: 202-214.

COON S.L. & KLEIN D.C., 2006. - Evolution of arylalkylamine N-acetyltransferase: emergence and divergence. *Mol. Cell. Endocrinol.*, 252: 2-10.

COON S.L., BÉGAY V., DEURLOO D., FALCÓN J. & KLEIN D.C., 1999. - Two arylalkylamine N-acetyltransferase genes mediate melatonin synthesis in fish. *J. Biol. Chem.*, 274: 9076-9082.

DECRESSAC S., GRECHEZ-CASSIAU A., LENFANT J. & BOIS P., 2002. - Cloning, localization and functional properties of a cGMP-gated channel in photoreceptor cells from fish pineal gland. *J. Pineal Res.*, 33(4): 225-233.

DUFOUR S., SEBERT M.E., WELTZIEN F.A., ROUSSEAU K. & PASQUALINI C., 2010. - Neuroendocrine control by dopamine of teleost reproduction. *J. Fish Biol.*, 76(1): 129-160.

EKSTRÖM P. & MEISSL H., 1997. - The pineal organ of teleost fishes. *Rev. Fish Biol. Fish.*, 7: 199-284.

EKSTRÖM P., VAN VEEN T., BRUUN A. & EHINGER B., 1987. - GABA-immunoreactive neurons in the photosensory pineal organ of the rainbow trout: two distinct neuronal populations. *Cell Tissue Res.*, 250: 87-92.

FALCÓN J., 1979. - The pineal organ of the pike, *Esox lucius* L. 1. Light and electron-microscopic study. *Ann. Biol. Anim., Bioch., Biophys./Reprod. Nutr. Dev.*, 19: 445-465.

FALCÓN J., 1999. - Cellular circadian clocks in the pineal. *Prog. Neurobiol.*, 58: 121-162.

FALCÓN J. & MEISSL H., 1981. - The photosensory function of the pineal organ of the pike (*Esox lucius*, L.). Correlation between structure and function. *J. Comp. Physiol.*, 144: 127-137.

FALCÓN J. & COLLIN J.P., 1985. - *In vitro* uptake and metabolism of [3H]indole compounds in the pineal organ of the pike. II. A autoradiographic study. *J. Pineal Res.*, 2(4): 357-373.

FALCÓN J., BALEMANS M.G., VAN BENTHEM J. & COLLIN J.P., 1985a. - *In vitro* uptake and metabolism of [3H]indole compounds in the pineal organ of the pike. I. A radiochromatographic study. *J. Pineal Res.*, 2(4): 341-356.

FALCÓN J., JUILLARD M.T. & COLLIN J.P., 1985b. - The pineal organ of the pike (*Esox lucius* L.). IV. Endogenous serotonin and monoamine-oxidase activity. A histochemical, ultra-cytochemical and pharmacological study. *Reprod. Nutr. Dev.*, 20(1A): 139-154.

FALCÓN J., GUERLOTTE J., VOISIN P. & COLLIN J.P., 1987. - Rhythmic melatonin biosynthesis in a photoreceptive pineal organ . A study in the pike. *Neuroendocrinology*, 45(6): 479-486.

FALCÓN J., BESSE C., GUERLOTTE J. & COLLIN J.P., 1988. - 5'-Nucleotidase activity in the pineal organ of the pike. An electron-microscopic study. *Cell. Tissue Res.*, 251: 495-502.

FALCÓN J., THIBAULT C., MARTIN C., BRUN-MARMILLON J., CLAUSTRAT B. & COLLIN J.P., 1991. - Regulation of melatonin production by catecholamines and adenosine in a photoreceptive pineal organ. An *in vitro* study in the pike and the trout. *J. Pineal Res.*, 11: 123-134.

FALCÓN J., BÉGAY V., BESSE C., RAVAULT J.P. & COLLIN J.P., 1992. - Pineal photoreceptor cells in culture: fine structure and light control of cyclic nucleotide levels. *J. Neuroendocrinol.*, 4: 641-651.

FALCÓN J., BÉGAY V., GOUJON J.M., VOISIN P., GUYERLOTTÉ J. & COLLIN J.P., 1994. - Immunocytochemical localization of hydroxyindole-O-methyltransferase in pineal photoreceptor cells of several fish species. *J. Comp. Neurol.*, 341(4): 559-566.

FALCÓN J., BOLLIET V. & COLLIN J.P., 1996a. - Partial characterization of serotonin N-acetyltransferases from northern pike (*Esox lucius*, L.) pineal organ and retina: effects of temperature. *Pflügers Arch.*, 432: 386-393.

FALCÓN J., MOLINA-BORJA M., COLLIN J.P. & OAKNIN S., 1996b. - Age-related changes in 2-[125I]-iodomelatonin binding sites in the brain of sea breams (*Sparus aurata*, L.). *Fish Physiol. Biochem.*, 15: 401-411.

FALCÓN J., GALARNEAU K.M., WELLER J.L., RON B., CHEN G., COON S.L. & KLEIN D.C., 2001. - Regulation of arylalkylamine N-acetyltransferase-2 (AANAT2, EC 2.3.1.87) in the fish pineal organ: evidence for a role of proteasomal proteolysis. *Endocrinology*, 142: 1804-1813.

FALCÓN J., BESSEAU L., FAZZARI D., ATTIA J., GAILDRAT P., BEAUCHAUD M. & BOEUF G., 2003. - Melatonin modulates secretion of growth hormone and prolactin by trout pituitary glands and cells in culture. *Endocrinology*, 144: 4648-4658.

FALCÓN J., BESSEAU L. & BOEUF G., 2007a. - Molecular and cellular regulation of pineal organ responses. *In:* Sensory Systems Neuroscience-Fish Physiology (Hara T. & Zielinski B., eds), pp. 243-406. Academic Press, Elsevier Inc.

FALCÓN J., BESSEAU L., SAUZET S. & BOEUF G., 2007b. - Melatonin effects on the hypothalamo-pituitary axis in fish. *Trends Endocrinol. Metab.*, 18: 81-88.

FALCÓN J., BESSEAU L., FUENTÈS M., SAUZET S., MAGNANOU E. & BOEUF G., 2009. - Structural and functional evolution of the pineal melatonin system in vertebrates. *Ann. NY Acad. Sci.*, 1163: 101-111.

FALCÓN J., MIGAUD H., MUÑOZ-CUETO J.A. & CARRILLO M., 2010. - Current knowledge on the melatonin system in teleost fish. *Gen. Comp. Endocrinol.*, 165(3): 469-482.

GAILDRAT P., RON B. & FALCÓN J., 1998. - Daily and circadian variations in 2-[125I]iodomelatonin binding sites in the pike brain (*Esox lucius*, L.). *J. Neuroendocrinol.*, 10: 511-517.

GAILDRAT P., BECQ F. & FALCÓN J., 2002. - First cloning and functional characterization of a melatonin receptor in fish brain: a novel one? *J. Pineal Res.*, 32:74-84.

GERN W.A. & GREENHOUSE S.S., 1988. - Examination of *in vitro* melatonin secretion from superfused trout (*Salmo gairdneri*) pineal organs maintained under diel illumination or continuous darkness. *Gen. Comp. Endocrinol.*, 71: 163-174.

GOTHILF Y., COON S.L., TOYAMA R., CHITNIS A., NAMBOODIRI M.A. & KLEIN D.C., 1999. - Zebrafish serotonin N-acetyltransferase-2: marker for development of pineal photoreceptors and circadian clock function. *Endocrinology*, 140: 4895-4903.

HENDERSON R.J., BELL M.V., PARK M.T., SARGENT J.R. & FALCÓN J., 1994. - Lipid composition of the pineal organ from rainbow trout (*Oncorhynchus mykiss*). *Lipids*, 29: 311-317.

HERRERA-PÉREZ P., DEL CARMEN RENDÓN M., BESSEAU L., SAUZET S., FALCÓN J. & MUÑOZ-CUETO J.A., 2010. - Melatonin receptors in the brain of the European sea bass: an *in situ* hybridization and autoradiographic study. *J. Comp. Neurol.*, 518: 3495-3511.

HIRAYAMA J., FUKUDA I., ISHIKAWA T., KOBAYASHI Y. & TODO T., 2003. - New role of zCRY and zPER2 as regulators of sub-cellular distributions of zCLOCK and zBMAL proteins. *Nucleic Acids Res.*, 31: 935-943.

IIGO M., KEZUKA H., SUZUKI T., TABATA M. & AIDA K., 1994. - Melatonin signal transduction in the goldfish, *Carassius auratus*. *Neurosci. Biobehav. Rev.*, 18: 563-569.

IIGO M., SANCHEZ-VAZQUEZ E.J., HARA M., OHTANI-KANEKO R., HIRATA K., SHINOHARA H., TABATA M. & AIDA K., 1997. - Characterization, guanosine 5'-O-(3-thiotriphosphate) modulation, daily variation, and localization of melatonin-binding sites in the catfish (*Silurus asotus*) brain. *Gen. Comp. Endocrinol.*, 108(1): 45-55.

IIGO M., ABE T., KAMBAYASHI S., OIKAWA K., MASUDA T., MIZUSAWA K., KITAMURA S., AZUMA T., TAKAGI Y., AIDA K. & YANAGISAWA T., 2007. - Lack of circadian regulation of *in vitro* melatonin release from the pineal organ of salmonid teleosts. *Gen. Comp. Endocrinol.*, 154(1-3): 91-97.

IKEGAMI T., AZUMA K., NAKAMURA M., SUZUKI N., HATTORI A. & ANDO H., 2009a. - Diurnal expressions of four subtypes of melatonin receptor genes in the optic tectum and retina of goldfish. *Comp. Biochem. Physiol A: Mol. Integr. Physiol.*, 152(2): 219-224.

IKEGAMI T., MOTOHASHI E., DOI H., HATTORI A. & ANDO H., 2009b. - Synchronized diurnal and circadian expressions of four subtypes of melatonin receptor genes in the diencephalon of a puffer fish with lunar-related spawning cycles. *Neurosci. Lett.*, 462(1): 58-63.

JAILLON O., AURY J.M., BRUNET F. *et al.* (61 authors), 2004. - Genome duplication in the teleost fish *Tetraodon nigroviridis* reveals the early vertebrate proto-karyotype. *Nature*, 431: 946-957.

KAHN I.A. & THOMAS P., 1996. - Melatonin influences gonadotropin II secretion in the Atlantic croaker (*Micropogonias undulatus*). *Gen. Comp. Endocrinol.*, 104: 231-242.

KEZUKA H., FURUKAWA K., AIDA K. & HANYU I., 1988. - Daily cycles in plasma melatonin levels under long or short photoperiod in the common carp, *Cyprinus carpio*. *Gen. Comp. Endocrinol.*, 72: 296-302.

KLEIN D.C., COON S.L., ROSEBOOM P.H., WELLER J.L., BERNARD M., GASTEL J.A., ZATZ M., IUVONE P.M., RODRIGUEZ I.R., BÉGAY V., FALCÓN J., CAHILL G.M., CASSONE V.M. & BALER R., 1997. - The melatonin rhythm-generating enzyme: molecular regulation of serotonin N-acetyltransferase in the pineal gland. *Recent Prog. Horm. Res.*, 52: 307-357.

KORF H.W., VON GALL C. & STEHLE J., 2003. - The circadian system and melatonin: lessons from rats and mice. *Chronobiol. Int.*, 20: 697-710.

KULCZYKOWSKA E., KALAMARZ H., WARNE J. & BALMENT R., 2006. - Day-night specific binding of 2-[I-125] iodomelatonin and melatonin content in gill, small intestine and kidney of three fish species. *J. Comp. Physiol. B*, 176(4): 277-285.

LOPEZ-OLMEDA J.F., OLIVEIRA C., KALAMARZ H., KULCZYKOWSKA E., DELGADO M.J. & SANCHEZ-VAZQUEZ F.J., 2009. - Effects of water salinity on melatonin levels in plasma and peripheral tissues and on melatonin binding sites in European sea bass (*Dicentrarchus labrax*). *Comp. Biochem. Physiol. A-Mol. Integr. Physiol.*, 52(4): 486-490.

LÓPEZ-OLMEDA J.F. & SÁNCHEZ-VÁZQUEZ F.J., 2010. - Feeding rhythms in fish: from behavioural to molecular approaches. *In*: Biological Clocks in Fish (Kulczykowska E., Popek W. & Kapoo B.D., eds), pp. 155-183. Enfield NH: CRC press.

LÓPEZ PATIÑO M.A., ALONSO-GÓMEZ A.L., GUIJARRO A., ISORNA E. & DELGADO M.J., 2008. - Melatonin receptors in brain areas and ocular tissues of the teleost *Tinca tinca*: characterization and effect of temperature. *Gen. Comp. Endocrinol.*, 155(3): 847-856.

MARTINEZ-CHAVEZ C.C. & MIGAUD H., 2009. - Retinal light input is required to sustain plasma melatonin rhythms in Nile tilapia *Oreochromis niloticus niloticus*. *Brain Res.*, 1269: 61-67.

MARTINEZ-CHAVEZ C.C., AL-KHAMEES S., CAMPOS-MENDOZA A., PENMAN D.J. & MIGAUD H., 2008. - Clock-controlled endogenous melatonin rhythms in Nile tilapia (*Oreochromis niloticus niloticus*) and African catfish (*Clarias gariepinus*). *Chronobiol. Int.*, 25:31-49.

MASUDA T., IIGO M., MIZUSAWA K., NARUSE M., OISHI T., AIDA K. & TABATA M., 2003. - Variations in plasma melatonin levels of the rainbow trout (*Oncorhynchus mykiss*) under various light and temperature conditions. *Zool. Sci.*, 20: 1011-1016.

MAX M. & MENAKER M., 1992. - Regulation of melatonin production by light, darkness, and temperature in the trout pineal. *J. Comp. Physiol. A*, 170: 479-489.

MAYER I., BORNESTAF C. & BORG B., 1997. - Melatonin in non-mammalian vertebrates: physiological role in reproduction? *Comp. Biochem. Physiol. A-Mol. Integr. Physiol.*, 118: 515-531.

MEISSL H. & EKSTRÖM P., 1988. - Photoreceptor responses to light in the isolated pineal organ of the trout, *Salmo gairdneri*. *Neuroscience*, 25(3): 1071-1076.

MEISSL H., ANZELIUS M., ÖSTHOLM T. & EKSTRÖM P., 1993. - Interaction of GABA, benzodiazepines and melatonin in the photosensory pineal organ of salmonid fish. *In*: Melatonin and the Pineal Gland: From Basic Science to Clinical Applications (Touitou Y., Arendt J. & Pévet P., eds), pp. 95-98. Amsterdam: Elsevier.

MEISSL H., YANEZ J., EKSTRÖM P. & GROSSMANN E., 1994. - Benzodiazepines influence melatonin secretion of the pineal organ of the trout *in vitro*. *J. Pineal Res.*, 17: 69-78.

MIGAUD H., DAVIE A., MARTINEZ CHAVEZ C.C. & AL-KHAMEES S., 2007. - Evidence for differential photic regulation of pineal melatonin synthesis in teleosts. *J. Pineal Res.*, 43: 327-335.

MIGAUD H., DAVIE A. & TAYLOR J.F., 2010. - Current knowledge on the photoneuroendocrine regulation of reproduction in temperate fish species. *J. Fish Biol.*, 76(1): 27-68.

MOLINA BORJA M., FALCÓN J., URQUIOLA E. & RAVAULT J.P., 1996 - Production of melatonin by the gilthead sea bream pineal: an *in vivo* and *in vitro* study. *Fish Physiol. Biochem.*, 15(5): 413-419.

MORTON D.J. & FORBES H.J., 1988. - Pineal gland N-acetyltransferase and hydroxyindole-O- methyltransferase activity in the rainbow trout (*Salmo gairdneri*): seasonal variation linked to photoperiod. *Neurosci. Lett.*, 94: 333-337.

MYERS B.R. & SIGAL Y.M. & JULIUS D., 2009. - Evolution of thermal response properties in a cold-activated TRP channel. *PloS ONE*, 4(5): e5741.

NIKAIDO Y., UEDA S. & TAKEMURA A., 2009. - Photic and circadian regulation of melatonin production in the Mozambique tilapia *Oreochromis mossambicus*. *Comp. Biochem. Physiol. A-Mol. Integr. Physiol.*, 152(1): 77-82.

OKIMOTO D.K. & STETSON M.H., 1999. - Presence of an intrapineal circadian oscillator in the Teleostean family Poeciliidae. *Gen. Comp. Endocrinol.*, 114(2): 304-312.

OLIVEIRA C. & SÁNCHEZ-VÁZQUEZ F.J., 2010. - Reproduction rhythms in fish. *In*: Biological Clocks in Fish (Kulczykowska E., Popek W. & Kapoor B.D., eds), pp. 185-215. Enfield, NH: CRC Press.

OLIVEIRA C., LÓPEZ-OLMEDA J.F., DELGADO M.J., ALONSO-GÓMEZ A.L. & SÁNCHEZ-VÁZQUEZ F.J., 2008. - Melatonin binding sites in Senegal sole: day/night changes in density and location in different regions of the brain. *Chronobiol. Int.*, 25(4): 645-652.

OLIVEIRA C., GARCIA E.M., LÓPEZ-OLMEDA J.F. & SÁNCHEZ-VÁZQUEZ F.J., 2009. - Daily and circadian melatonin release *in vitro* by the pineal organ of two nocturnal teleost species: Senegal sole (*Solea senegalensis*) and tench (*Tinca tinca*). *Comp. Biochem. Physiol. A-Mol. Integr. Physiol.*, 153(3): 297-302.

OMURA Y. & OGURI M., 1969. - Histological studies on the pineal organ of 15 species of teleost fishes. *Bull. Jpn. Soc. Fish.*, 35: 991-1000.

OMURA Y., KORF H.W. & OKSCHE A., 1985. - Vascular permeability (problem of the blood-brain barrier) in the pineal organ of the rainbow trout, *Salmo gairdneri*. *Cell Tissue Res.*, 239: 599-610.

PARK Y.J., PARK J.G., HIYAKAWA N., LEE Y.D., KIM S.J. & TAKEMURA A., 2007a. - Diurnal and circadian regulation of a melatonin receptor, MT1, in the golden rabbitfish, *Siganus guttatus*. *Gen. Comp. Endocrinol.*, 150: 253-262.

PARK Y.J., PARK J.G., JEONG H.B., TAKEUCHI Y., KIM S.J., LEE Y.D. & TAKEMURA A., 2007b. - Expression of the melatonin receptor Mel1c in neural tissues of the reef fish *Siganus guttatus*. *Comp. Biochem. Physiol. A-Mol Integr Physiol.*, 147(1): 103-111.

PAUL M.J., ZUCKER I. & SCHWARTZ W.J., 2008. - Tracking the seasons: the internal calendars of vertebrates. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 363(1490): 341-361.

PING Y. HUANG H., ZHANG X.J. & YANG X.L., 2008. - Melatonin potentiates rod signals to ON type bipolar cells in fish retina. *J. Physiol.*, 586: 2683-2694.

POPEK W., LUSZCZEK-TROJNAR E., DRAG-KOZAK E., FORTUNA-WRONSKA D. & EPLER P., 2005. - Effect of the pineal gland and melatonin on dopamine release from perifused hypothalamus of mature female carp during spawning and winter regression. *Acta Ichthyol. Piscat.*, 35: 65-71.

POPEK W., LUSZCZEK-TROJNAR E., DRAG-KOZAK E., RZASA J. & EPLER P., 2006. - Effect of melatonin on dopamine secretion in the hypothalamus of mature female common carp, *Cyprinus carpio* L. *Acta Ichthol. Piscat.*, 36: 135-141.

POPEK W., DRAG-KOZAK E. & LUSZCZEK-TROJNAR E., 2010. - Influence of melatonin on hypothalamo-pituitary-ovarian axis in carp; mechanisms of action. *In*: Biological Clocks in Fish (Kulczykowska E., Popek W. & Kapoor B.D., eds), pp. 217-34. Enfield, NH: CRC Press.

RAVI V. & VENKATESH B., 2008. - Rapidly evolving fish genomes and teleost diversity. *Curr. Opi. Gen. Dev.*, 18(6): 544-50.

RIBELAYGA C., WANG Y. & MANGEL S.C., 2004. - A circadian clock in the fish retina regulates dopamine release via activation of melatonin receptors. *J. Physiol.*, 554: 467-482.

REITER R.J., TAN D.X. & MANCHESTER L.C., 2010. - Melatonin in fish: circadian rhythms and functions. *In*: Biological Clocks in Fish. Kulczykowska E., Popek W. & Kapoor B.D., eds), pp. 71-9. Enfield, NH: CRC Press.

SANCHEZ-VAZQUEZ F.J., IIGO M., MADRID J.A. & TABATA M., 2000. - Pinealectomy does not affect the entrainment to light nor the generation of the circadian demand feeding rhythms of rainbow trout. *Physiol. Behav.*, 69:455-461.

SANTINI F., HARMON L.J., CARNEVALE G. & ALFARO M.E., 2009. - Did genome duplication drive the origin of teleosts? A comparative study of diversification in ray-finned fishes. *BMC Evol. Biol.*, 9: 194.

SATO T.K., PANDA S., MIRAGLIA L.J., REYES T.M., RUDIC R.D., MCNAMARA P., NAIK K.A., FITZGERALD G.A., KAY S.A. & HOGENESCH J.B., 2004. - A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. *Neuron*, 43: 527-537.

SAUZET S., BESSEAU L., HERRERA PEREZ P., COVÈS D., CHATAIN B., PEYRIC E., BOEUF G., MUÑOZ-CUETO J.A. & FALCÓN J., 2008. - Cloning and retinal expression of melatonin receptors in the European sea bass, *Dicentrarchus labrax*. *Gen. Comp. Endocrinol.*, 157(2): 186-195.

SEBERT M.E., LEGROS C., WELTZIEN F.A., MALPAUX B., CHEMINEAU P. & DUFOUR S., 2008. - Melatonin activates brain dopaminergic systems in the eel with an inhibitory impact on reproductive function. *J. Neuroendocrinol.*, 20(7): 917-929.

SEEBACHER F. & MURRAY S.A., 2007. - Transient receptor potential ion channels control thermoregulatory behaviour in reptiles. *PLoS ONE*, 2: e281.

SETH M. & MAITRA S.K., 2010. - Importance of light in temporal organization of photoreceptor proteins and melatonin-producing system in the pineal of carp *Catla catla*. *Chronobiol. Int.*, 27(3): 463-486.

SIMONNEAUX V. & RIBELAYGA C., 2003. - Generation of the melatonin endocrine message in mammals: a review of the complex regulation of melatonin synthesis by norepinephrine, peptides, and other pineal transmitters. *Pharmacol. Rev.*, 55: 325-395.

SKÖLD H., AMUNDSEN T., SVENSSON P.A., MAYER I., BJELVENMARK J. & FORSGREN E., 2008. - Hormonal regulation of female nuptial coloration in a fish. *Horm. Behav.*, 54(4): 549-556.

SMITH A. TRUDEAU V.L., WILLIAMS L.M., MARTINOLI M.G. & PRIEDE I.G., 1996. - Melatonin receptors are present in non-optic regions of the brain of a deep-sea fish living in the absence of solar light. *J. Neuroendocrinol.*, 8: 655-658.

SOENGAS J.L. & ALDEGUNDE M., 2002. - Energy metabolism of fish brain. *Comp. Biochem. Physiol. B*, 131(3): 271-296.

STRAND J.E.T., AARSETH J.J., HANEBREKKE T.L. & JØRGENSEN E., 2008. - Keeping track of time under ice and snow in a sub-arctic lake: plasma melatonin rhythms in Arctic charr overwintering under natural conditions. *J. Pineal Res.*, 44(3): 27-233.

THIBAULT C., FALCÓN J., GREENHOUSE S.S., LOWERY C.A., GERN W.A. & COLLIN J.P., 1993. - Regulation of melatonin production by pineal photoreceptor cells: role of cyclic nucleotides in the trout (*Oncorhynchus mykiss*). *J. Neurochem.*, 61: 332-339.

UNDERWOOD H., 1989. - The pineal and melatonin: regulators of circadian function in lower vertebrates. *Experientia*, 45: 914-922.

VALLONE D., GONDI S.B., WHITMORE D. & FOULKES N.S., 2004. - E-box function in a period gene repressed by light. *Proc. Nat. Acad. Sci. USA*, 101: 4106-111.

VERA L.M., DE OLIVEIRA C., LOPEZ-OLMEDA J.F., RAMOS J., MANANOS E., MADRID J.A. & SANCHEZ-VAZQUEZ F.J., 2007. - Seasonal and daily plasma melatonin rhythms and reproduction in Senegal sole kept under natural photoperiod and natural or controlled water temperature. *J. Pineal Res.*, 43(1): 50-55.

VUILLEUMIER R., BESSEAU L., BOEUF G., PIPARELLI A., GOTHILF Y., GEHRING W.G., KLEIN D.C. & FALCÓN J., 2006. - Starting the zebrafish pineal circadian clock with a single photic transition. *Endocrinology*, 147(5): 2273-2279.

YAÑEZ J. & MEISSL H., 1995. - Secretion of methoxyindoles from trout pineal organs *in vitro*: indication for a paracrine melatonin feedback. *Neurochem. Int*., 27: 195-200.

ZACHMANN A., KNIJFF S.C.M., ALI M.A. & ANCTIL M., 1992. - Effects of photoperiod and different intensities of light exposure on melatonin levels in the blood, pineal organ and retina of the brook trout (*Salvelinus frontinalis*, Mitchill). *Can. J. Zool*., 70: 25-29.

ZILBERMAN-PELED B., BENHAR I., COON S.L., RON B. & GOTHILF Y, 2004. - Duality of serotonin-N-acetyltransferase in the gilthead seabream (*Sparus aurata*): molecular cloning and characterization of recombinant enzymes. *Gen. Comp. Endocrinol*., 138: 139-147.

ZILBERMAN-PELED B., APPELBAUM L., VALLONE D., FOULKES N.S., ANAVA S., ANZULOVICH A., COON S.L., KLEIN D.C., FALCÓN J., RON B. & GOTHILF Y., 2007. - Transcriptional regulation of arylalkylamine-N-acetyltransferase-2 gene in the pineal gland of the gilthead seabream. *J. Neuroendocrinol*., 19: 46-53.

ZIV L., LEVKOVITZ S., TOYAMA R., FALCÓN J. & GOTHILF Y., 2005. - Functional development of the zebrafish pineal gland: light-induced expression of period2 is required for onset of the circadian clock. *J. Neuroendocrinol*., 17: 314-320.

Reçu le 10 septembre 2010.
Accepté pour publication le 14 février 2011.